

# Stability of fatty acid monolayers and the relationship between equilibrium spreading pressure, phase transformations, and polymorphic crystal forms

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**ABSTRACT** Force-area isotherms were obtained for hexadecanoic, octadecanoic, eicosanoic, and docosanoic acid monolayers at different compression rates. Equilibrium spreading pressures were determined both by monolayer collapse and by spreading from the bulk phase. Monolayers formed metastable phases at all pressures above their equilibrium spreading pressures and at all surface areas smaller than the surface areas at their equilibrium spreading pressures. These metastable phases collapsed to stable phases at the equilibrium spreading pressures of the fatty acids. Collapse phenomena and compression experiments at very slow compression rates suggested that a previously unrecognized phase transformation occurred at the equilibrium spreading pressure. The surface area at this phase transformation corresponded to the cross-sectional area of the C-form of the fatty acid crystal. Fatty acid monolayers at the phase transformations previously described by other workers had surface areas which were closely related to molecular areas in their A and B polymorphic crystal forms. These correlations indicated that molecular structure in saturated fatty acid monolayers was similar to molecular structure in fatty acid crystals.

**SUPPLEMENTARY KEY WORDS** hexadecanoic · octadecanoic · eicosanoic · docosanoic · compression rate · collapse · surface area · molecular structure

**T**HE STABILITY of a fatty acid monolayer affects many properties of the monolayer particularly in the measurement of the surface pressure and surface area of phase transformation points such as the collapse pressure of the film (1). Stability has not been studied in detail by many

investigators although Ries and Kimball (2) obtained electron micrographs which suggested that a stearic acid monolayer collapsed into a bilayer during compression. Rabinovitch, Robertson, and Mason (3) later found that the compression rate had a profound effect on the shape of the force-area isotherm since stearic acid films were compressed to smaller areas when the films were compressed at slower rates. Since the collapse of metastable films into bilayers and multilayers could explain the disappearance of films maintained at a constant surface pressure (4), we have reinvestigated the surface properties of saturated fatty acids and determined both the shape of the force-area isotherms which are obtained at very slow compression rates and the stability of monolayers maintained at surface pressures below and above their equilibrium spreading pressures. Stability data suggest that the equilibrium spreading pressure represents a previously unrecognized phase transformation in the monolayer which may be correlated with one polymorphic crystal form of the fatty acid.

## MATERIALS AND METHODS

Fatty acids were purchased from Applied Science Laboratories Inc. (State College, Pa.). *n*-Hexane was first washed with concentrated sulfuric acid and then was refluxed for 12hr with 5% potassium permanganate. After successive washes with 5% sodium bisulfite, 5% sodium bicarbonate, and saturated sodium chloride, the hexane was passed through anhydrous sodium sulfate and finally distilled at 69°C. Water was doubly distilled

from glass into polyethylene containers and had a specific conductance of  $1.6 \times 10^{-6}$  mho.

Surface pressure ( $\pi$ ) was measured by the Wilhelmy plate technique (5) utilizing a Cahn R. G. recording balance and a Varian model 20 recorder. The Langmuir trough (inner dimensions  $1 \times 9.8 \times 50$  cm), was milled from a solid Teflon block. A platinum foil 1 cm wide was used as the dipping plate. The movable bar was milled from Teflon and was propelled by a high-torque variable speed motor. The trough and weighing assembly were enclosed in a plexiglass case. Fatty acids, dissolved in hexane, were spread on 0.1 N hydrochloric acid. All measurements were made at the ambient temperature.

## RESULTS

The force-area ( $\pi$ -A) isotherms were generated at a number of different compression rates. Continuous compression at rates which varied between 0.5 and 6.0  $\text{\AA}^2$  per molecule per min had little effect on the transition point (T), the collapse point (C), and the shape of the isotherm in the precollapse region of the curve (Fig. 1). Very slow compression rates were achieved by intermittent compression and equilibration, and this compression method had a profound effect on the shape of the  $\pi$ -A isotherm (Fig. 2).  $\pi$  increased during compression, and  $\pi$  decreased during equilibration. Both the increase and decrease in  $\pi$  were directly proportional to the chain length of the fatty acid.

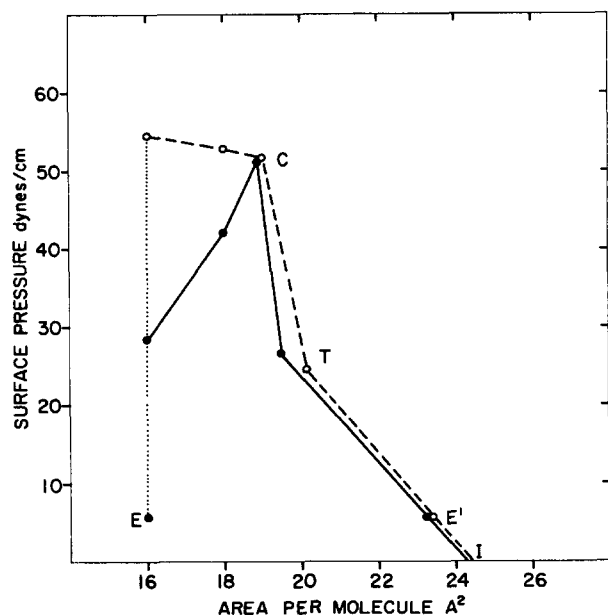


FIG. 1.  $\pi$ -A isotherms for eicosanoic acid spread on 0.1 N hydrochloric acid at 25–27°C. Monolayers were compressed at 0.62  $\text{\AA}^2$  per molecule per min (●) and at 5.93  $\text{\AA}^2$  per molecule per min (○).

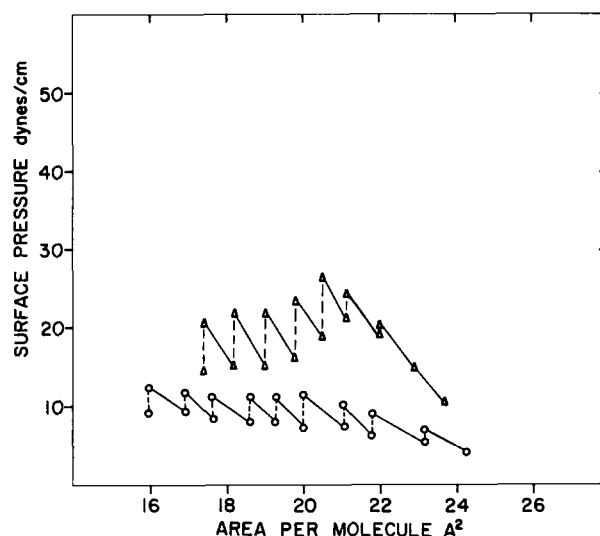


FIG. 2.  $\pi$ -A isotherms for hexadecanoic acid (○) and docosanoic acid (Δ) monolayers during intermittent compression and equilibration. Hexadecanoic acid was compressed for 2 min at 0.46  $\text{\AA}^2$  per molecule per min and then equilibrated for 20 min. Docosanoic acid was compressed for 2 min at 0.4  $\text{\AA}^2$  per molecule per min and then equilibrated for 20 min. Monolayers were spread on 0.1 N hydrochloric acid, and the temperature was 31°C.

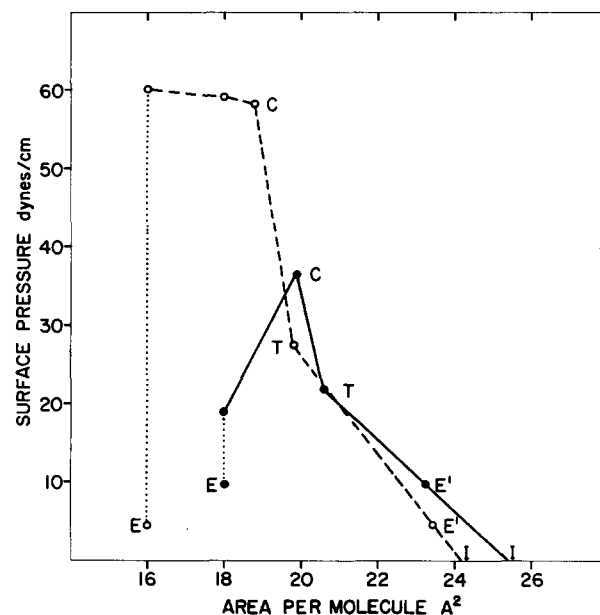


FIG. 3.  $\pi$ -A isotherms for hexadecanoic acid (●) and docosanoic acid (○) spread on 0.1 N hydrochloric acid at 25–27°C. Monolayers were compressed at rates which varied from 0.5  $\text{\AA}^2$  per molecule per min to 6.0  $\text{\AA}^2$  per molecule per min. Data represent the mean values obtained for compression at different rates, since the shape of the curve in the postcollapse region was not affected by the compression rate.

The shape of the  $\pi$ -A isotherm in the postcollapse region of the curve depended on both the compression rate and the chain length of the fatty acid.  $\pi$  decreased rapidly after collapse when an eicosanoic acid film was compressed slowly, and  $\pi$  increased somewhat after col-

lapse when the film was compressed rapidly (Fig. 1). Eicosanoic acid was a transition compound. The post-collapse  $\pi$  for hexadecanoic and octadecanoic acid monolayers decreased rapidly when the film was compressed at either a rapid or slow rate (Fig. 3). Conversely, the postcollapse  $\pi$  for docosanoic acid monolayers remained constant or increased when the film was compressed at either a slow or rapid rate (Fig. 3).

When compression was stopped at any point beyond *C*,  $\pi$  decreased rapidly for a few minutes and then decreased slowly over a 12–18 hr period until it reached a constant and reproducible value (Figs. 1 and 3). This  $\pi$  was specific for each fatty acid, and it appeared that hexadecanoic and octadecanoic acid monolayers collapsed to  $\pi$  values which were nearly equal to the equilibrium spreading pressures ( $\pi_E$ ) that have been reported for these acids when they were spread from excess bulk phases (6, 7).  $\pi_E$  values were then determined experimentally by spreading from the crystalline state with the four acids used in this study. The monolayers were spread from bulk phases placed at the air-water interface of the Langmuir trough, and  $\pi$  was monitored until equilibrium was attained. The  $\pi_E$  values were similar to the  $\pi$  values obtained after monolayer collapse and equilibration (Table 1).

Monolayer collapse at lower  $\pi$  values had been noted when films were compressed at very slow rates (Fig. 2). Subsequent experiments showed that monolayers collapsed to  $\pi_E$  when compression was stopped at any pressure greater than  $\pi_E$  although the rate of collapse from films at pressures slightly greater than  $\pi_E$  was much slower than the rate of collapse from films in the high pressure or postcollapse region of the isotherm. Thus  $\pi$  decreased about 35 dynes/cm in 1 min when compression was stopped in the postcollapse region of eicosanoic and docosanoic acid monolayers (Figs. 1 and 3), while  $\pi$  decreased only 7.5 dynes/cm during the 20-min equilibration periods in a slow compression and equilibration sequence for the docosanoic acid film (Fig. 2).

## DISCUSSION

Correlations between molecular structure in fatty acid monolayers and molecular structure in fatty acid crystals were first suggested by Müller (8), Lyons and Rideal (9), and Dervichian (10). Dervichian noted similarities between surface areas at specific phase transformations, the cross-sectional area reported for the B-form of stearic acid (8) and cross-sectional areas which he calculated for the A- and C-forms of stearic acid. However, the surface area data obtained by Dervichian contained small errors, and consequently, his hypothesis was not generally accepted (11). Other hypotheses which involved intermolecular hydrogen bonding (12), the inhibition of chain rotation (13), and changes in the conformations of hydroxyl groups (14) were suggested as explanations for phase transformations in fatty acid monolayers even though Stållberg-Stenhagen and Stenhagen (15), Stenhagen (16), and Lundquist (17) were able to relate different phases of a docosanoic acid monolayer to different packing modes for hydrocarbon crystals.

Fatty acid monolayers exhibit three phase transformations during their compression at room temperature (11, 15–17). The  $L_2$  phase appears at the initial or limiting area, point *I* on the  $\pi$ -A isotherm, and this phase is transformed to the LS phase when the film is compressed to point *T* on the isotherm (Figs. 1 and 3). The LS to S phase transformation occurs between points *T* and *C* on the isotherm, and the area at *C* is the smallest area at which the monolayer exists in the S phase. An additional low pressure phase, the  $L'_2$  phase, exists in eicosanoic and docosanoic acid monolayers, but this phase is not observed unless the temperature of the monolayer is lowered below room temperature (15–17).

The S and LS phases are metastable states. The  $L_2$  phase has both stable and metastable regions. Monolayers in the S and LS phases are transformed spontaneously to the lower pressure and larger area  $L_2$  phase, and this phase is stable at  $\pi_E$ . Brooks and Alexander (18) in a study of fatty alcohol monolayers predicted that the

TABLE 1 SURFACE AREA AND SURFACE PRESSURE AT THE PHASE TRANSFORMATIONS OF SATURATED FATTY ACID MONOLAYERS SPREAD ON 0.1 N HYDROCHLORIC ACID AT 25–27°C

Transformation Property	Fatty Acid			
	16:0	18:0	20:0	22:0
$A_I^*$	25.4 $\pm$ 0.6	24.7 $\pm$ 0.6	24.4 $\pm$ 0.1	24.2 $\pm$ 0.7
$A_E$	22.8 $\pm$ 0.4	23.1 $\pm$ 0.6	23.2 $\pm$ 0.2	23.4 $\pm$ 0.7
$\pi_E$ (Collapse)	9.7	7.8	5.4	4.4
$\pi_E$ (Spreading)	10.0	7.3	3.9	3.4
$A_T$	20.6 $\pm$ 0.3	20.3 $\pm$ 0.7	19.7 $\pm$ 0.3	19.8 $\pm$ 0.5
$\pi_T$	22.0 $\pm$ 0.4	22.0 $\pm$ 1.2	25.9 $\pm$ 0.8	27.6 $\pm$ 1.8
$A_C$	19.9 $\pm$ 0.2	19.2 $\pm$ 0.6	19.0 $\pm$ 0.1	18.8 $\pm$ 0.5
$\pi_C$	36.5 $\pm$ 2.9	52.0 $\pm$ 1.7	51.5 $\pm$ 0.4	58.7 $\pm$ 0.7

\*  $A$  is the surface area,  $\text{\AA}^2$  per molecule  $\pm$  sd.

†  $\pi$  is the surface pressure, dynes/cm  $\pm$  sd. Subscripts indicate transformation points on the  $\pi$ -A isotherm (Figs. 1 and 3).

monolayers were in a metastable state after  $\pi_E$  and that these monolayers would collapse. The effect of monolayer collapse on the shape of the compression isotherm has been described by Rabinovitch, Robertson, and Mason (3). These investigators found that an octadecanoic acid monolayer could not be compressed to the collapse point when the film was compressed at a slow rate or when the film was repeatedly compressed and decompressed. Our studies suggest that a phase transformation occurs at  $\pi_E$  when hexadecanoic acid is compressed at a very slow rate (Fig. 2). Fatty acids with longer chains such as docosanoic acid may be compressed somewhat beyond  $\pi_E$  and do not collapse to  $\pi_E$  during the slow equilibration period (Fig. 2). The absence of a transformation at  $\pi_E$  for docosanoic acid during slow and intermittent compression suggests that the collapse rate for this fatty acid is much slower than the collapse rate for hexadecanoic acid. This transformation should be observed by a slower compression rate or a longer equilibration between intermittent compressions. The shape of the curve in the post-collapse region of the compression isotherm also suggests that the collapse rate depends on the chain length of the fatty acid. Thus hexadecanoic acid films always show a decrease in  $\pi$  at slow compression rates (Fig. 3), and docosanoic acid films do not show a decrease in  $\pi$  even at slow compression rates (Fig. 3). It is apparent that the collapse of fatty acid monolayers may be compensated by compression, and this compensation explains why the low pressure phase transformation at  $\pi_E$  is not observed when monolayers are compressed at the usual rates.

A relationship between molecular structure in monolayers and bulk phases was suggested by Stållberg-Stenhagen and Stenhagen (15) who showed that the surface area for docosanoic acid in the S phase at the collapse point was similar to the cross-sectional area obtained by x-ray diffraction for hydrocarbons in the lower symmetry or triclinic phase (19, 20). They reasoned from these data that the lateral packing for the hydrocarbon chains of docosanoic acid in the S phase corresponded to the lateral packing for hydrocarbons in the triclinic crystal. In a later study, von Sydow (21) showed that the A-form of crystalline dodecanoic acid had the same structure as the triclinic hydrocarbon. Surface areas at the collapse point for all the fatty acids examined in our study ( $A_C$  in Table 1) are very similar to the cross-sectional areas for hydrocarbons in the triclinic phase, about  $19.6 \text{ \AA}^2$  per molecule. Thus, all fatty acid monolayers in the S phase appear to have highly ordered structures which are closely related to their crystal structures in the A-form.

Hydrocarbon chains for fatty acid monolayers in the LS phase are generally described as vertical and capable of both rotational and translational motion (15–17).

A vertical orientation was suggested because the surface area of a molecule in the LS phase is only slightly larger than the surface area of the molecule in the S phase. However, the surface areas of fatty acids at the point where they are transformed into the LS phase ( $A_T$  in Table 1) are very similar to the cross-sectional areas obtained by x-ray diffraction for the tilted B-forms of these fatty acids. The cross-sectional areas obtained by x-ray diffraction are  $20.8 \text{ \AA}^2$  per molecule for the B-form of hexadecanoic acid (22) and  $20.5\text{--}20.7 \text{ \AA}^2$  per molecule for the B-form of octadecanoic acid (8, 23). The correlations between surface area and the molecular area in a crystal lattice suggest that the fatty acid monolayers in the LS phase have highly ordered structures which are closely related to their tilted crystal structures in the B-form.

Both Stenhagen (16) and Lundquist (17) have suggested that hydrocarbon chains are tilted in the lower pressure and larger area  $L'_2$  and  $L_2$  phases. The surface properties of enantiomorphs and racemic mixtures in the  $L_2$  phase indicate that the phases have considerable molecular structure, and liquid-to-solid transformations within these phases have been postulated (16, 17). The  $\pi_E$  data support this hypothesis. Surface areas of fatty acids at  $\pi_E$  ( $A_E$  in Table 1) are very similar to the cross-sectional areas obtained by x-ray diffraction for the tilted C-forms of these fatty acids. Cross-sectional areas are  $23.8 \text{ \AA}^2$  per molecule for the C-form of hexadecanoic acid (22),  $23.2 \text{ \AA}^2$  per molecule for the C-form of octadecanoic acid (24), and  $23.0 \text{ \AA}^2$  per molecule for the C-form of docosanoic acid (24). The correlations between surface area at  $\pi_E$  and the molecular area in a crystal form could be explained by a liquid-to-solid transformation at  $\pi_E$  where the monolayer exists as a solid phase with a molecular structure of the C-form of the fatty acid crystal. With rapid compression, the monolayer passes through the C, B, and A packing modes before it collapses into the stable C conformation. With slow or intermittent compression, sufficient time is available for the monolayer to collapse toward the C conformation during the compression experiment.

The data and explanations presented in this study support the early hypothesis of Müller (8), Lyons and Rideal (9), and Dervichian (10) that molecular structure in saturated fatty acid monolayers is very similar to molecular structure in the different polymorphic forms of saturated fatty acid crystals. Saturated phospholipids exhibit polymorphism (25), and phase transformations have been observed in monolayers of saturated phospholipids (26–29). Unsaturated phospholipids with *trans* double bonds exhibit both polymorphism and a monolayer phase transformation (30). Membrane lipids are largely unsaturated and monolayers of these unsaturated fatty acids have large surface areas and do not



exhibit phase transformations. Chapman, Owens, Phillips, and Walker (31) recently suggested that cholesterol reduced the molecular motion in the fatty acyl side chains of phospholipids. If cholesterol effectively reduces molecular motion, the phospholipid-cholesterol mixtures in membranes may have more highly ordered structures than was previously supposed, and phase transformations may be an important property of natural membranes.

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